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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No. 4250-2
 First Inventor or Application Identifier Robert H. Keller, M.D.
 Title "Method of Treatment of Glutathione..."
 Express Mail Label No. EJ339415165US

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. ☒ * Fee Transmittal Form (e.g., PTO/SB/17)
 (Submit an original and a duplicate for fee processing)
2. ☒ Specification [Total Pages 24]
 (preferred arrangement set forth below)
 - Descriptive title of the invention
 - Cross References to Related Applications
 - Statement Regarding Fed sponsored R & D
 - Reference to Microfiche Appendix
 - Background of the invention
 - Brief Summary of the invention
 - Brief Description of the Drawings (if filed)
 - Detailed Description
 - Claim(s)
 - Abstract of the Disclosure
3. ☐ Drawing(s) (35 U.S.C. 113) [Total Sheets ☐
4. ☒ Oath or Declaration [Total Pages 3]
 - a. ☒ Newly executed (original or copy)
 - b. ☐ Copy from a prior application (37 C.F.R. § 1.63(d))
 (for continuation/divisional with Box 17 completed)
 (Note Box 5 below)
 - c. ☐ DELETION OF INVENTOR(S)
 Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).
5. ☐ Incorporation By Reference (useable if Box 4b is checked)
 The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered to be part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

ADDRESS TO:

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6. ☐ Microfiche Computer Program (Appendix)
7. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
 - a. ☐ Computer Readable Copy
 - b. ☐ Paper Copy (identical to computer copy)
 - c. ☐ Statement verifying identity of above copies

ACCOMPANYING APPLICATION PARTS

8. ☒ Assignment Papers (cover sheet & document(s))
9. ☐ 37 C.F.R. §3.73(b) Statement (when there is an assignee) ☐ Power of Attorney
10. ☐ English Translation Document (if applicable)
11. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
12. ☐ Preliminary Amendment
13. ☒ Return Receipt Postcard (MPEP 503)
 (Should be specifically itemized)
14. ☒ * Small Entity Statement(s) ☐ Statement filed in prior application, Status still proper and desired (PTO/SB/09-12)
15. ☐ Certified Copy of Priority Document(s) (if foreign priority is claimed)
16. ☒ Other: check for \$420.00

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17. If a CONTINUING APPLICATION, check appropriate box, and supply the requisite information below and in a preliminary amendment

☐ Continuation ☐ Divisional ☐ Continuation-in-part (CIP) of prior application No: _____

Prior application information: Examiner _____

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18. CORRESPONDENCE ADDRESS

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Express Mail # EJ339415165US

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9(f) AND 1.27 (c)) - SMALL BUSINESS CONCERN			Docket No. 4250-2
Serial No.	Filing Date herewith	Patent No.	Issue Date
Applicant/ Robert H. Keller, M.D. and David W. Kirshenbaum Patentee:			
Invention: Method of Treatment of Glutathione Deficient Mammals			
<p>I hereby declare that I am:</p> <p><input checked="" type="checkbox"/> the owner of the small business concern identified below:</p> <p><input type="checkbox"/> an official of the small business concern empowered to act on behalf of the concern identified below:</p> <p>NAME OF CONCERN: <u>VIT-IMMUNE, L.C.</u></p> <p>ADDRESS OF CONCERN: <u>5821 Hollywood Boulevard, Hollywood, Florida 33021</u></p> <p>I hereby declare that the above-identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.</p> <p>I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the above identified invention described in:</p> <p><input checked="" type="checkbox"/> the specification filed herewith with title as listed above.</p> <p><input type="checkbox"/> the application identified above.</p> <p><input type="checkbox"/> the patent identified above.</p> <p>If the rights held by the above-identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed on the next page and no rights to the invention are held by any person, other than the inventor, who could not qualify as an independent inventor under 37 CFR 1.9(c) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).</p>			

Express Mail # EJ339415165US

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

- ☒ no such person, concern or organization exists.
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Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING:

RH KELLER MD

TITLE OF PERSON SIGNING

OTHER THAN OWNER:

ADDRESS OF PERSON SIGNING: 5821 Hollywood Boulevard
Hollywood, Florida 33021

SIGNATURE:

RH Keller MD

DATE:

4/29/99

METHOD OF TREATMENT OF GLUTATHIONE DEFICIENT MAMMALS

BACKGROUND OF THE INVENTION

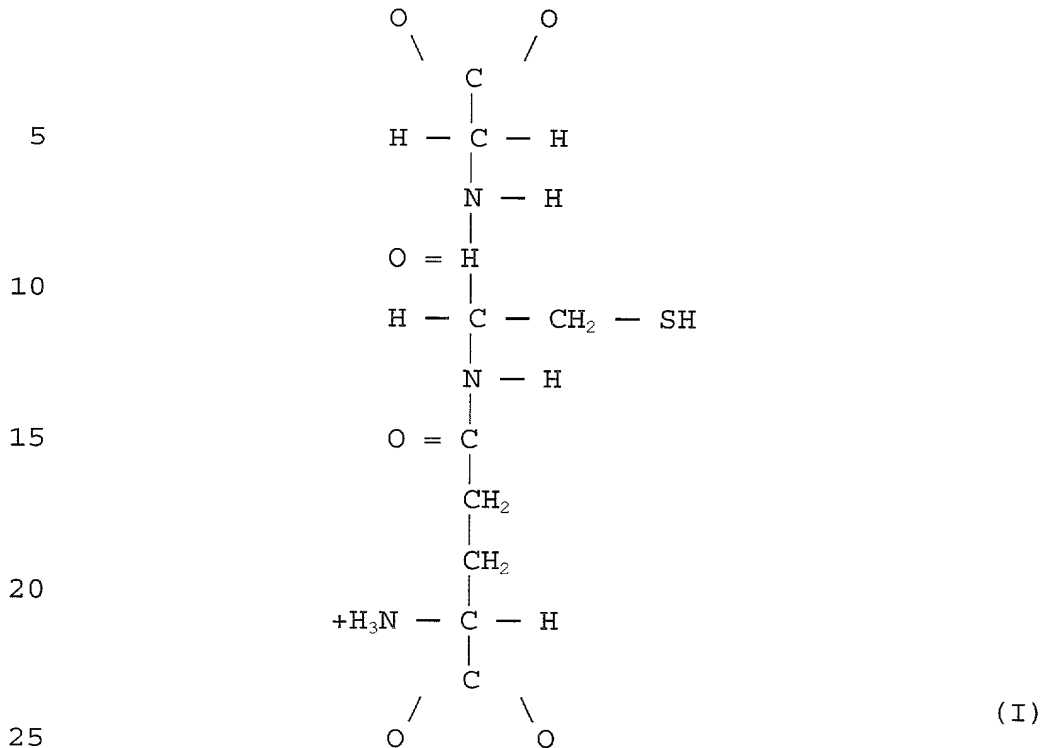
Field of the Invention

This invention provides a method of improving
5 glutathione (GSH) concentrations, both intra and
extra-cellularly, in mammals, thereby improving the
cellular and humoral immune response. It comprises
oral administration of a therapeutically effective
amount of nutritional supplement which is composed of
10 critical and synergistic quantities of amino acids,
peptides, and bioflavanoids.

Brief Description of Related Art

Glutathione is a well-known tripeptide, which
exists in two basic forms. The antioxidant form or
15 "reduced glutathione" tripeptide is conventionally
called "glutathione" and abbreviated as "GSH". The
oxidized form is a sulfur-sulfur linked compound known
as glutathione disulfide (GSSG).

Glutathione in its biologically active, reduced
20 form (GSH) has the formula:



and is appropriately named γ -L-Glutamyl-L-cysteinylglycine. It is ubiquitous in animals, plants, and microorganisms and being water soluble is found mainly in the cell cytosol and other aqueous phases of the living system. Glutathione often attains millimolar levels inside living cells, which makes it one of the most highly concentrated intracellular antioxidants.

Glutathione is homeostatically controlled, both inside the animal cell and outside. Enzyme systems synthesize it, utilize it, and regenerate it per the gamma-glutamyl cycle. (Meister A. Glutathione, Ascorbate, and Cellular Protection Cancer Res (Suppl) 1994 (Apr 1); 54:1969S-1975S).

Glutathione is most concentrated in the mammal liver (10mM), where the P450 Phase II" enzymes require it to convert fat-soluble substances into water-soluble GSH conjugates in order to facilitate their excretion.

- 5 While providing GSH for their specific needs, the liver parenchymal cells export GSH to the outside, where it serves as systemic source of—SH/reducing power.

Briefly, glutathione synthesis occurs within
10 animal cells in two closely linked enzymatically controlled reactions that utilize Adenosine Triphosphate (ATP) and draw on nonessential amino acids as substrates. First, cysteine and glutamate are combined (by the enzyme gamma-glutamyl cysteinyl
15 synthetase, with availability of cysteine usually being the rate- limiting factor. Cysteine is generated from the essential amino acid methionine, from the degradation of dietary protein, or from turnover of endogenous proteins. The buildup of GSH
20 acts to feedback-inhibit this enzyme, thereby helping to ensure homeostatic control over GSH synthesis.

The second GSH synthesis reaction combines gamma-glutamylcysteine with glycine to generate GSH (catalyzed by GSH synthetase).

- 25 With regard to the essentiality of GSH for the survival of the mammal, substantial information is available from studies on hereditary GSH depletion in the human, and from experimental depletion and repletion of GSH in animal models and cell cultures,
30 see for example: Meister A. Larsson A. Glutathione Synthetase Deficiency and Other Disorders of the Gamma-Glutamyl Cycle; Sriver CR. et al eds. The

Metabolic and Molecular Bases of Inherited Disease (Volume I). New York: McGraw-Hill: 1995:1461-1495 (Chapter 43); and Beutler E. Nutritional and Metabolic Aspects of Glutathione, Annu Rev Nutr 1989;9:287-302.

5 Reduced GSH levels in mammalian cells are associated with a wide variety of pathophysiologic states, including hepatic dysfunction, malignancies, HIV infection, pulmonary disease, Parkinson's disease, related immunologic illnesses and physiological
10 conditions; see for example the descriptions in Kidd, Alternative Medicine Review, Vol. 2, No. 3, pages 156-176 (1997).

 The consequences of sustained GSH depletion are fatal. As cellular GSH is depleted, first individual
15 cells die in those areas most affected. Then zones of tissue damage begin to appear. Localized free-radical damage spreads across the tissue in an ever-widening, self-propagating wave.

 An object of this invention is to promote
20 gastrointestinal absorption and intracellular uptake of components which will maximize intracellular reduced glutathione production by a mammal including a human.

Summary of the Invention

25 The invention comprises a composition of matter, which comprises in admixture:

 N-acetylcysteine;
 vitamin C;and
 a pharmaceutically acceptable systemic carrier
30 for oral administration.

 In preferred embodiments, the invention further comprises one or more of the following:

alpha-lipoic acid;
sylmarin;
quercitin;
l-glutamine;
5 N-acetyl-d-glucosamine;
a probiotic.

The invention also comprises systemic administration of the composition of the invention to a mammal suffering from low glutathione levels, to
10 stimulate the natural production of glutathione in the biological cells of the mammal.

The term "low glutathione levels" as used herein means a blood glutathione level below about 440 μg glutathione/ 10^{10} erythrocytes, determined by the
15 colorimetric method of Beutler et al., Improved Method for the Determination of Blood Glutathione, J. Lab. Clin. Med., 61;882-8(1963). Normal levels in humans ranges from about 440 to 654 $\mu\text{g}/10^{10}$ erythrocytes.
Detailed Description Of The Preferred
20 Embodiments Of The Invention

Recently, there have been many scientific papers published discussing the direct relationship between decreased glutathione levels and the progression of many chronic diseases. Glutathione functions as an
25 antioxidant, antitoxin and protector of red blood cells, and is extremely important to the immune system. It neutralizes free radicals minimizing the damage they cause and is profoundly important for cellular homeostasis.

30 As with other cell types, the proliferation, growth, and differentiation of immune cells is dependent on GSH. Both the T and the B lymphocytes

5 Effect of glutathione depletion and oral N-acetyl-
cysteine treatment on CD4+ and CD8+ cells. FASEB J
1994;8:448-451. Intracellular GSH is also required
for the T-cell proliferative response to mitogenic
stimulation, for the activation of cytotoxic T
0 "killer" cells, and for many specific T-cell
functions, including DNA synthesis for cell
replication, as well as for the metabolism of
interleukin-2 which is important for the mitogenic
response; Wu D. Meydani SN, Sastre J. et al. In-
5 vitro glutathione supplementation enhances
interleukin-2 production and mitogenic response of
peripheral blood mononuclear cells from young and old
subjects; J Nutr 1994;124:655-663.

In summary, it has been demonstrated that decreased levels of glutathione may be a result of various types of prolonged stress, increased free radical formation and hyperactivity of the immune system. These factors in turn compromise the health of mammalian cells. Despite the apparent importance of adequate glutathione levels, little emphasis has heretofore been placed on replacing depleted stores. Some glutathione comes from the diet but the majority is made in the liver.

Studies have demonstrated that oral glutathione
30 supplementation is not well absorbed by many of the
mammal's cells and does not replenish losses inside
cells where it is most needed; Witschi A. Reddy S.

Stofer B. et al. The systemic availability of Oral Glutathione. Eur. J Clin. Pharmacol. 1992;43:667-669.

The sulfur-containing amino acid l-cysteine is the precursor that most limits the cellular biosynthesis of GSH. When substituted into the diet in place of the total protein allowance it was effective in raising GSH levels (see Witschi et al., supra.).

Glutathione esters, synthetic compounds prepared by linking the glycol end of GSH into ester bonds, have been the subject of much research by Meister, Anderson, supra., as potential oral GSH delivery compounds (see also U.S. Patent 4,784,685). These esters do appear to be effective GSH delivery vehicles, but have the disadvantage that they yield alcohols in vivo when their ester bonds are broken, and their safety over the long term has yet to be satisfactorily demonstrated.

We have discovered that to efficiently raise the level of glutathione intracellularly, it is necessary to employ several different mechanisms that work simultaneously. First, essential elements needed by the body for the manufacture of glutathione must be introduced. Second, gastro-intestinal health of the mammal must be optimal to facilitate nutrient absorption. Third, the liver function must be supported and protected as the liver is the glutathione "manufacturing and storage house". Lastly, recycling existing glutathione and enhancing enzymatic reactions that promote glutathione synthesis are also important functions which are advantageous to support.

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As mentioned above, support of liver function in the mammal being treated for low glutathione levels is

advantageous. For this purpose, there may be orally administered to the mammal the following:

A. Sylmarin serves to improve and restore liver function. It quenches free radicals, reduces potential toxicity, and stimulates protein synthesis necessary to create new liver cells. Also known as "silibin", "silybin" or "silybinin", Sylmarin is a generic term for extract from the mature fruits of *Silybum marianum* (sometimes *Carduus marianus*), commonly known as milk thistle; see Madaus AG publication: Legalon. Koln, Germany, 1989 and Valenzuela A, et al. Sylmarin Protection Against Hepatic Lipin Peroxidation Induced by Acute Ethanol Intoxication in Rats, *Biochemical Pharmacology*, 1985:34(12):2209-2212. Sylmarin is available under the trade name Legalon®, from Madaus AG, (Jarrow Formulas, Inc.; Madaus, 1989).

B. Quercetin [2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one] is used for its ability to eliminate toxic compounds found in the liver. It has anti-hepatotoxic, antiviral, anti-inflammatory and antibacterial properties. It may be synthesized by the method of Shakhova et al., *Zh. Obsheh. Khim.*, 32, 390 (1962).

Advantageously, the following nutritional are also employed in the method of the invention.

L-glutamine is an essential dietary component for the support of gastrointestinal growth and function and it is utilized as fuel in the small intestines. It is used by the intestinal tract in large amounts for energy during periods of physiological stress. It has been shown to preserve liver glutathione after

lethal hepatic injury and nourish tissues in the GI tract, liver and immune system, see for example; Souba, W.W., et al. The Role of Glutamine in Maintaining a Healthy Gut and Supporting the Metabolic Response to Injury and Infection. J. Of Surgical Res., 990:48(4): 83-91.

N-acetyl-d-glucosamine (NAG) is a key precursor in the biosynthesis of mucosal glycoproteins that form glycocalyx. The glycocalyx is the most superficial, highly viscous layer of the gut mucosa that comes in contact with intestinal contents. The glycoprotein layer acts to protect the underlying tissues from exposure to enzymes, acid and bacterial assault while providing a selectively absorptive surface, Wilmore, D.W., et al, The gut: a Central Organ After Surgical Stress; Surgery 1988: 104, (5):917-23.

Probiotics or "healthy bacteria" are necessary as they breakdown nutrients, eliminate toxins and inhibit harmful bacteria that enter mammalian systems through the GI tract. The term "Probiotic" is defined herein as "A live microbial food supplement which beneficially affects the host mammal by improving its microbial balance". Representative of healthy bacteria are isolates of bifidobacteria, lactobacilli, such as Lactobacillus acidophilus and Lactobacillus casei, propionibacteria, and enterococci. Lactobacilli are preferred in the composition and method of the invention (see Perdigon, G. et al., Immunology 63:17-23 (1988)). More preferably Lactobacillus rhamnosus, Lactobacillus casei, Bifidobacterium longum, Bifidobacterium infantis, Lactobacillus acidophilus, and Saccharomyces boulardi are used.

Finally, a source of dietary protein is preferred and advantageous to supplement the nutritional needs of the mammal. We have found that the compositions of the invention and the method herein described are
5 optimized by inclusion of a biologically active whey protein composition comprising an undenatured whey protein concentrate obtained from raw mammalian milk. This concentrate contains substantially all of the heat labile whey protein found in the raw milk.
10 Representative of concentrate which are commercially available include Promod™, available from Ross Laboratories, Division of Abbott Laboratories, Chicago, Illinois. Concentrates may also be prepared by the method described in U.S. Patent 5,290,571,
15 incorporated herein by reference thereto. The undenatured whey protein concentrates also contain a rich variety of immunoglobulins which boast the immunologic response of the mammal treated with the concentrates; see for example U.S. Patent 5,456,924
20 which is incorporated herein by reference thereto.

A high protein, low fat whey has immuno-supportive properties. It is rich in naturally active immunoglobulins, essential amino acids and other important nutrients critical for proper nutrient
25 utilization within the gut.

We have discovered that the ingredients described above work synergistically to provide the necessary nutrients required for glutathione production while supporting the mammal's ability to produce and
30 preserve existing stores of GSH. The effect of the admixture of ingredients is far more significant than the individual ingredients alone.

This invention also relates also to pharmaceutical dosage unit forms for systemic administration (oral, topical administration) which are useful in treating mammals, including humans.

- 5 The term "dosage unit form" as used in this specification and in the claims refers to physically discrete units suitable as unitary dosage for mammalian subjects, each unit containing a predetermined quantity of the essential active
- 10 ingredient; calculated to produce the desired effect in combination with the required pharmaceutical means which adapt said ingredient for systemic administration. Examples of dosage unit forms in accordance with this invention are tablets, capsules,
- 15 orally administered liquid preparations in liquid vehicles, suppositories, and dry preparations for the extemporaneous preparation of preparations in a liquid vehicle. Solid diluents or carriers for the solid oral pharmaceutical dosage unit forms are selected
- 20 from the group consisting of lipids, carbohydrates, proteins and mineral solids, for example, starch, sucrose, kaolin, dicalcium phosphate, gelatin, acacia, corn syrup, corn starch, talc and the like. Capsules, both hard and soft, are formulated with conventional
- 25 diluents and excipients, for example, edible oils, talc, calcium carbonate, calcium stearate, magnesium stearate and the like. Liquid pharmaceutical preparations for oral administration may be prepared in water or aqueous solutions which advantageously
- 30 contain suspending agents, such as for example, sodium carboxymethylcellulose, methylcellulose, acacia, polyvinyl pyrrolidone, polyvinyl alcohol and the like.

Such preparations must be stable under the conditions of manufacture and storage, and ordinarily contain in addition to the basic solvent or suspending liquid, preservatives in the nature of bactericidal and fungicidal agents, for example, parabens, chlorobutanol, benzyl alcohol, phenol, thimerosal, and the like. In many cases it is preferable to include isotonic agents, for example, sugars or sodium chloride. Carriers and vehicles include vegetable oils, water, ethanol, and polyols, for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like.

The pharmaceutical dosage unit forms are prepared in accordance with the preceding general description to provide an effective amount of the essential active ingredient per dosage unit form in admixture with the means for adaptation to systemic administration. In general, the unit dose form will contain 3 to 73 percent by weight of the essential active ingredient.

It will be appreciated that the exact dosage of the essential active ingredient constituting an effective amount for treatment of a mammal according to the method of the invention will vary greatly depending on the specific nature of the clinical condition being treated, severity of the condition, species of mammal, age, weight and condition of the mammal, mode of administration of the dosage form and the specific formulation being administered. The exact dose required for a given situation may be determined by administration of a trial dose and observation of the clinical response. In general, an effective amount to be administered

will be within a range of from about 0.1 mg. per kg.
to about 50 mg. per kg. of body weight of the
recipient, daily. Preferably 0.5 mg./kg. to about 25
mg./kg. daily is provided. In most instances, a
5 single month of administration will effect a
noticeable response and bring about the result
desired. In cases such as the treatment
of immunological conditions however, it may be
desirable to repeat the administrations several times
10 daily over longer periods of time.

The following examples and preparations describe
the manner and process of making and using the
invention and set forth the best mode contemplated by
the inventor of carrying out the invention but are not
15 to be construed as limiting.

Example 1

A mixture of the following ingredients is
prepared by hand mixing:

	<u>Ingredient</u>	<u>Quantity</u>
20	N-acetylcysteine	<u>1,000</u> to <u>20,000</u> mg
	vitamin C	<u>5,000</u> to <u>50,000</u> mg
	alpha-lipoic acid	<u>100</u> to <u>2,500</u> mg
	sylmarin	<u>100</u> to <u>2,500</u> mg
	Quercetin	<u>100</u> to <u>2,500</u> mg
25	l-glutamine	<u>500</u> to <u>2,000</u> mg
	N-acetyl-d-glucosamine	<u>500</u> to <u>2,000</u> mg
	whey protein concentrate	<u>1,000</u> to <u>20,000</u> mg

Lactobacillus acidophilus
Twenty Million to One Billion
30 CFU; Schiff Products, Inc.,
Salt Lake City, Utah.

orange essence flavor Adjust to taste

The mixture which constitutes the essential

active ingredient of a preferred embodiment of the invention, together with a flavorant may be compounded into wafers, tablets or capsules containing 750 to 14,000 mg of active ingredient. In an uncompounded form, the powder dry mixture may be orally administered to a human (one teaspoonful, once or twice daily) as a dietary supplement or as recommended by a health care professional. Alternatively, the dry powder may be mixed with juice, water or food to facilitate administration.

Example 2

Three dosage units in powder form, each containing 500 mg of essential active ingredient (e.g. an amount of the mixture of Example 1, supra) were prepared from the following ingredients:

essential active ingredient	1500 g
starch (Rx-1500)	300 g
magnesium stearate, USP	39 g
colloidal silicic acid	19.5 g
Avicel ® pH 102. q.s. to	3900 g

The essential active ingredient was ground through a 0.25 mm sieve opening screen. The powdered active ingredient, with 50% of the total amount of magnesium stearate be used, colloidal silicic acid and Avicel ® pH 10.2 were passed through a 40 mesh sieve, mixed for 20 minutes and then slugged. The slugs were broken down by forcing through a screen No. 11, and mixed with the remaining magnesium stearate.

One dosage given orally 1-4 times a day is useful in the relief of immuno-deficiency in adult humans provoked by infective disease, or other etiological causes.

Example 3

Three thousand dosage units for oral use, each containing 750 mg of the essential active ingredient, were prepared from the following ingredients:

5	essential active ingredient	750 g
	colloidal silicic acid	30 g
	magnesium stearate USP	30 g
	microcrystalline cellulose	150 g
	lactose	90 g

- 10 In accordance with the active ingredient potency, the amount of lactose was adjusted to achieve a weight of 900 mg for each dosage unit. The ingredients were passed through a 40 mesh sieve and mixed for 30 minutes. The powder may be mixed into a drink or
- 15 inserted into hard gelatin capsules No. 0 and filled using Zanazi, model RV-59 equipment. The capsules should be preserved in airtight, light-resistant containers.

- When administered to a human adult suffering from
- 20 low levels of glutathione (GSH) 1 to 4 dosage units daily, the level is adjusted upward to a normal range.

Example 4

A mixture of the following ingredients is formed into a powdered dosage form in the following

- 25 proportion:

	Ingredient	Quantity
	Vitamin C (ascorbate)	1000 mg
	N-acetylcysteine	1500 mg
	L-Glutamine	3000 mg
30	N-acetyl d-glucosamine	500 mg
	Alpha Lipoic acid (ALA)	75 mg
	Quercetin	75 mg

	Sylimarine	100 mg
	Whey protein	500 mg
	Conjugated linoleic acid (CLA)	500 mg
	Orange flavor	380 mg
5	Rice syrup	1516 mg
	Malic acid	7.8 mg
	Citric acid	7.8 mg
	Stevia	455 mg
	GI Balance (Probiotic)	300 mg

10 Our studies have shown that the administration of
the above dosage unit (one rounded teaspoon) mixed
into a liquid 1-4 times (preferably 2 times) a day is
useful in the relief of immuno-deficiency in adult
humans provoked by infective disease, or other
15 etiological causes. For example, the inventive
composition can be used effectively to improve
hepatic function e.g. decreased inflammation (ALT) in
patients with chronic hepatitis C and patients who are
receiving protease inhibitors as part of HAART therapy
20 for HIV. Both groups demonstrated an increase in
intra lymphocyte GSH levels after the administration
of the inventive composition.

Our studies have shown systemic administration of
the composition results in an improvement in T
25 lymphocyte function which correlates directly with an
increased intra lymphocyte GSH. In addition, our data
demonstrates that the inventive composition and method
shifts the T-cell balance from TH2 (allergy producing)
to TH1 (viral/tumor killing) and the increases intra
30 lymphocyte GSH correlate directly with decreased
levels of IgE the immunoglobulin associated with

allergies. Further studies have revealed the following:

Systemic administration of the composition increases natural killer cell function which is
5 considered a primitive first line of cellular immune defense.

Systemic administration of the composition decreases serum cholesterol and triglycerides of between 10 and 20% in patients with a variety of
10 hyperlipidemias and a decrease in myalgias associated with illness and exercise and improved muscle recovery after exercise.

Systemic administration of the composition decreases fatigue in patients suffering from a variety
15 of illnesses including but not limited to chronic viral infections, HIV, hepatitis C, chronic fatigue, immuno deficiency syndrome, immune deficiencies, cancer, B-cell malignancies, including lymphomas, chronic leukemia, myeloma Waldenstrom's and MGUS.
20 This makes the composition function as both a pharmaceutical and a therapeutic substance for patients suffering from the debilitating conditions.

Initial studies have shown that the systemic administration of the inventive composition also
25 increases energy in people without illness who are exposed to increased stress.

As such, the combination formulated will improve hepatic function in conditions associated with chronic viral infections, as well as any condition associated
30 with increased hepatic work or stress.

Thus by the present invention its advantages will be realized and although preferred embodiments have

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been disclosed and described in detail herein, its scope should not be limited thereby rather its scope should be determined by that of the appended claims.

[illegible]

CLAIMS

1. A composition of matter, which comprises in admixture;
N-acetylcysteine;
vitamin C whereby the amount of vitamin C is in an amount sufficient to facilitate the absorption of N-acetylcysteine across the cellular membrane; and
a pharmaceutically acceptable systemic carrier for oral administration.
2. The composition of claim 1 further comprising one or more of the following substances from the group consisting of alpha-lipoic acid, sylmarin, quercitin, l-glutamine, N-acetyl-d-glucosamine, a probiotic, and dietary protein.
3. The composition of claim 1 further comprising alpha-lipoic acid, sylmarin, quercitin, l-glutamine, N-acetyl-d-glucosamine, and a probiotic.
4. The composition of claim 3 further comprising dietary protein.
5. The composition of claim 1 further comprising flavorants.
6. The systemic administration of a pharmaceutically effective amount of the composition according to claim 1 to a mammal suffering from low glutathione levels, to stimulate the natural

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[illegible]

7. The systemic administration of a pharmaceutically effective amount of the composition according to claim 2 to a mammal suffering from hepatitis, to stimulate the natural production of glutathione in the biological cells of the mammal.
8. The systemic administration of a pharmaceutically effective amount of the composition according to claim 2 to a mammal suffering from HIV, to stimulate the natural production of glutathione in the biological cells of the mammal.
9. The systemic administration of a pharmaceutically effective amount of the composition according to claim 2 to a mammal suffering from allergies, to shift the T-cell balance from TH2 to TH1 and decrease levels of TgE.
10. The systemic administration of a pharmaceutically effective amount of the composition according to claim 2 to a mammal to decrease serum cholesterol and triglycerides.
11. The systemic administration of a pharmaceutically effective amount of the composition according to claim 2 to a mammal suffering from one or more of the following illnesses from the group consisting of chronic viral infections, HIV, hepatitis C, chronic fatigue, immuno deficiency syndrome,

immune deficiencies, cancer, B-cell malignancies, including lymphomas, chronic leukemia, myeloma Waldenstrom's and MGUS to decrease fatigue.

12. The systemic administration of a pharmaceutically effective amount of the composition according to claim 2 to a mammal to decrease fatigue.
13. The systemic administration of a pharmaceutically effective amount of the composition according to claim 2 to a mammal to decrease the effects of stress.
14. The systemic administration of a pharmaceutically effective amount of the composition according to claim 2 to a mammal to increase energy.
15. Administration according to claim 6 wherein a pharmaceutically effective is 0.1 mg/kg to about 50 mg/kg of body weight of the mammal, daily.
16. Administration according to claim 6 wherein a pharmaceutically effective is 0.5 mg/kg to about 25 mg/kg of body weight of the mammal, daily.
17. The systemic administration of a pharmaceutically effective amount of the composition according to claim 2 to a mammal suffering from low glutathione levels, to stimulate the natural production of glutathione in the biological cells of the mammal.

18. The systemic administration of a pharmaceutically effective amount of the composition according to claim 3 to a mammal suffering from low glutathione levels, to stimulate the natural production of glutathione in the biological cells of the mammal.

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Figure 1 consists of 12 sub-graphs labeled (a) through (l), each showing a different parameter over a 24-hour period. The parameters are: (a) Body weight (g), (b) Food intake (g), (c) Water intake (ml), (d) Urine volume (ml), (e) Urine osmolality (mOsm/kg), (f) Urine pH, (g) Urine creatinine (mg/dl), (h) Urine urea (mg/dl), (i) Urine sodium (mmol/l), (j) Urine potassium (mmol/l), (k) Urine calcium (mmol/l), and (l) Urine magnesium (mmol/l). Each graph shows a mean value with standard error of the mean (SEM) bars. The data points are connected by lines, showing trends over time. For example, body weight (a) decreases steadily over the 24 hours, while food intake (b) and water intake (c) show significant fluctuations, with water intake peaking around 12 hours. Urine volume (d) and osmolality (e) also show significant changes, with osmolality peaking around 12 hours. Urine pH (f) remains relatively stable around 7. Urine creatinine (g) and urea (h) show a slight increase over time. Urine sodium (i) and potassium (j) show a slight decrease over time. Urine calcium (k) and magnesium (l) show a slight increase over time.

Glutathione (GSH) is a tripeptide of extreme importance as a catalyst, reductant, and reactant. It can be depleted intracellularly either by forming a direct complex with an electrophilic agent (accomplished investigationaly by agents such as bromobenzene or diethyl maleate), by way of inhibition of synthesis, or by subjecting cells to oxidant stress. Most cells, except for epithelia cells, do not have a direct transport capacity for intact GSH. Non-epithelial cells must either transport precursor substrates for GSH synthesis or salvage amino acids from circulating GSH for reuse in intracellular resynthesis. Dietary cysteine is a rate limiting substrate for the synthesis of glutathione and also inhibits GSH efflux. Although GSH is synthesized from precursors in virtually all cells, the liver is the main source of plasma GSH. Protection and support of liver function is paramount to elevating GSH levels. The disclosure is also of a unique combination of nutritional supplements including n-acetyl cysteine, vitamin C, l-glucosamine, n-acetyl d-glucosamine, quercitin, sylimarin, Alpha lipoic acid and high protein, low fat whey that are combined to support various bodily systems involved in glutathione synthesis, reutilization and storage; all intended to elevate glutathione concentration in the mammalian cell.

(PCT/US-99 Pub. 605)

FORM 1-10.1

1-75

Approved for use through 9/30/98

PTO/SB/01 (8-95)

OMB 0651-0032

Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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Rev. 9/95U.S. Department of Commerce
Patent and Trademark Office

Attorney Docket Number

4250-2

First Named Inventor

Robert R. Keller, M.D.

COMPLETE IF KNOWN

Application Number

Filing Date

Group Art Unit

Examiner Name

DECLARATION FOR
UTILITY OR DESIGN
PATENT APPLICATION☒ Declaration Submitted with Initial Filing ☐ Declaration Submitted after Initial Filing

As a below named inventor, I hereby declare that:

My residence, postal office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD OF TREATMENT OF GLUTATHIONE DEFICIENT MAMMALS

(Title of the Invention)

The specification of which

☒ is attached hereto
OR☐ was filed on (MM/DD/YYYY)

as United States Application Number or PCT International

Application Number

and was amended on (MM/DD/YYYY)

(if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37 Code of Federal Regulations §1.56

I hereby claim foreign priority benefits under Title 35, United States Code §119 (a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365 (a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	<input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority sheet attached hereto.

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.
60/083, 661	04/30/98	

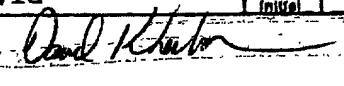
Burden Hour Statement: This form is estimated to take 4 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

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DECLARATION				Page 2	
<p>I hereby claim the benefit under Title 35, United States Code §120 of any United States application(s), or §363(c) of any PCT International application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of the application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which becomes available between the filing date of the prior application and the national or PCT International filing date of this application.</p>					
U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)		
<input type="checkbox"/> Additional U.S. or PCT International application numbers are listed on a supplemental sheet attached hereto.					
<p>As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office concerning this application:</p>					
<div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> Firm Name </div> <div>Customer Number or Issue </div> </div>					
<input type="checkbox"/> List attorney(s) and/or agent(s) name and registration number below:					
Name	Registration Number	Name	Registration Number		
Joseph C. Sullivan	18,720	Ronald R. Santucci	28,988		
John Kurucz	18,688	Ronald E. Brown	32,200		
Gerald Levy	24,419	John P. Gulbin	33,180		
Joseph T. Eisele	25,331	Richard J. Danyko	33,672		
Monami Roy	40,982	Clifford Ulrich	42,194		
<input type="checkbox"/> Additional attorney(s) and/or agent(s) named on a supplemental sheet attached hereto.					
<p>Please direct all correspondence to: <input type="checkbox"/> Customer Number OR <input checked="" type="checkbox"/> Fill in correspondence address below</p>					
Name Ronald R. Santucci					
Address Kane, Dalsimer, Sullivan, Kurucz, Levy, Eisele and Richard, LLP					
Address 711 Third Avenue, 20th Floor					
City New York,		State NY		ZIP 10017	
Country U.S.A.		Telephone 212-687-6000		Fax 212-682-3484	
<p>I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.</p>					
<p>Name of Sole or First Inventor: <input type="checkbox"/> A petition has been filed for this unsigned inventor</p>					
Given Name	Robert	Middle Initial	B	Family Name	Keller
					Suffix M.D.
Inventor's Signature					Date 4/19/97
Residence: City Weston		State FL Country U.S.A.		Citizenship U.S.A.	
Post Office Address 501 Ranch Road					
Post Office Address 					
City Weston	State FL	Zip 33326	Country U.S.A.		Applicant Authority
<input checked="" type="checkbox"/> Additional inventors are being named on supplemental sheet(s) attached hereto					

(Declaration for Utility or Design Patent Application (PTO/SB/01) [1-10.1]—page 2 of 3)

DECLARATION						ADDITIONAL INVENTOR(S) Supplemental Sheet	
Name of Additional Joint Inventor, if any:						<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name	David	Middle Initial	W	Family Name	Kirshenbaum	Suffix e.g., Jr.	
Inventor's Signature					Date	4/29/99	
Residence: City	Weston	State	FL	Country	U.S.A.	Citizenship	U.S.A.
Post Office Address		3272 Huntington					
Post Office Address							
City	Weston	State	FL	Zip	33332	Country	U.S.A.
						Applicant Authority	
Name of Additional Joint Inventor, if any:						<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name		Middle Initial		Family Name		Suffix e.g., Jr.	
Inventor's Signature					Date		
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		Zip		Country	
						Applicant Authority	
Name of Additional Joint Inventor, if any:						<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name		Middle Initial		Family Name		Suffix e.g., Jr.	
Inventor's Signature					Date		
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		Zip		Country	
						Applicant Authority	
Name of Additional Joint Inventor, if any:						<input type="checkbox"/> A petition has been filed for this unsigned inventor	
Given Name		Middle Initial		Family Name		Suffix e.g., Jr.	
Inventor's Signature					Date		
Residence: City		State		Country		Citizenship	
Post Office Address							
Post Office Address							
City		State		Zip		Country	
						Applicant Authority	

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